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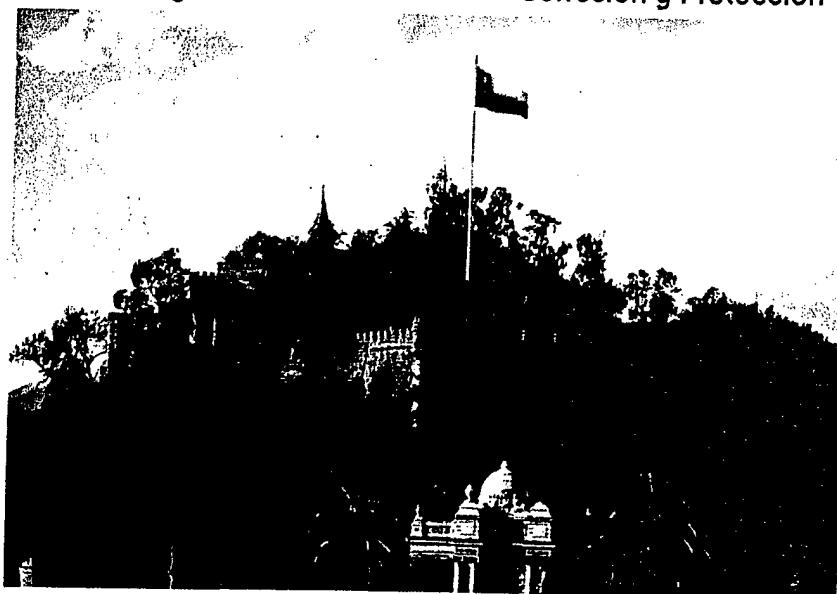
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A Comparison of Biotic and Inorganic Sulfide Films

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ABSTRACT

Distribution, tenacity and chemical composition of sulfide films produced by bacteria within biofilms were compared with those produced by waterborne inorganic sulfides. Attempts were made to differentiate corrosion mechanisms of alloy 400 (70Ni-30Cu) exposed to seawater in the presence or absence of sulfate-reducing bacteria (SRB). Experiments were conducted in an anaerobic environment in the presence of inorganic sulfide and SRB either freely corroding or coupled to an external cathode (alloy 400) exposed in aerobic media. Sulfur concentration in corrosion products increased in the presence of SRB and/or an external cathode. Bacteria encrusted with corrosion products and integrated into the sulfide film were only observed in the presence of SRB.

Keywords: microbiologically influenced corrosion, nickel-copper alloys, sulfate reducing bacteria, sulfide attack

INTRODUCTION

Determination of specific mechanisms for corrosion due to microbiologically mediated oxidation and reduction of sulfur is complicated by (1) the variety of potential metabolic/energy sources and by-products, (2) the coexistence of reduced and oxidized sulfur species, (3) competing reactions with inorganic and organic compounds, and (4) the versatility and adaptability of microorganisms. The physical scale over which the sulfur cycle influences corrosion varies with environment. The complete sulfur cycle of oxidation and reduction reactions can take place in macro (bulk) environments, including sewers and polluted harbours or within the microenvironment of biofilms.

Most of the literature on sulfide induced corrosion of copper and nickel alloys does not differentiate between corrosion due to waterborne sulfides and sulfides produced by SRB within biofilms. The problem of accelerated corrosion of copper/nickel and nickel/copper alloys by waterborne sulfides was identified in the 1970s and early 1980s.¹⁻³ In most cases, investigators used laboratory experiments in which 90/10 or 70/30 copper/nickel alloys were exposed to artificial or natural seawater with sodium sulfide. Gudas and Hack⁴ demonstrated that inorganic sulfide films enhanced galvanic corrosion under some circumstances. Syrett^{5,7} demonstrated that deaerated seawater containing dissolved inorganic sulfides did not immediately lead to accelerated corrosion. However, exposure of a porous sulfide corrosion product to oxygen produced aggressive corrosion. Investigators now recognize that most failures of copper and nickel alloys in actual seawater service are related to *in situ* sulfide production by SRB in biofilms.

Nickel/copper alloy 400 (Monel 400), nominally containing 66.5% nickel, 31.5% copper and 1.25% iron, is used for seawater and brackish water handling because of its resistance to chloride-ion stress corrosion cracking and erosion corrosion. Friend⁸ established that nickel/copper alloys containing more than 30% nickel formed a passive film similar to that formed on pure nickel. Localized corrosion of alloy 400 in seawater service is related to stagnation and/or intermittent flow.^{9,10}

EXPERIMENTAL

Disk shaped alloy 400 coupons (1.58 cm diameter x 0.158 cm thick) were purchased with an as-mill finish (Metal Samples, Munford, Alabama, USA). Wire leads (100 cm long) were electrically attached to the back of the samples by carbon tape and silver adhesive. Samples were embedded in EpoThin epoxy (Buehler Ltd, Lake Bluff, IL, USA). The epoxy created a watertight seal at the connections. Samples were wet-polished to a 1200 grit finish, sonicated in acetone for 5 minutes, rinsed with ethanol, and dried with nitrogen gas.

The mixed SRB species inoculum has been described previously.¹⁰⁻¹² All isolates are positive for desulfovordin (characteristic of *Desulfovibrio sp.*). 100-ml stock cultures of SRB were maintained in liquid growth medium (Postgate B)¹³ supplemented with NaCl (3% w/v). Cultures were kept in glass bottles fitted with rubber septa and aluminium crimped tops and were placed in glass canisters with an anaerobic gas generating system (BBL™ Gas Pak Plus™; Becton Dickinson Co., Sparks, MD) at 30 °C until use. Dissolved sulfide (S^{2-}) was determined by the methylene blue method¹⁴ using a Hach Model DR/2500 spectrophotometer (Hach Co., Loveland, CO).

Three 1.5 litre glass containers were filled with 1350 ml of artificial seawater (ASW) (35 ppt salinity, pH = 8.2). 150 ml of Postgate B medium was also added to make a suitable environment for bacterial growth. Containers were labelled 'SRB,' 'uninoculated,' and 'aerobic.' SRB and uninoculated containers were maintained in an anaerobic hood after purging for 15 minutes with nitrogen gas. Two alloy 400 samples in the vertical orientation were placed in each container (Figure 1). Three samples were similarly placed in the container containing aerobic media. A single saturated calomel reference electrode (SCE) was placed inside the anaerobic chamber in a beaker of saturated KCl solution. KCl salt bridges were used for continuous solution conductivity between containers. Each electrode was connected to a data logger that measured the corrosion potential vs. SCE every 10 minutes. No attempt was made to insure sterile conditions in the uninoculated control. The anaerobic chamber was maintained at 30 °C.

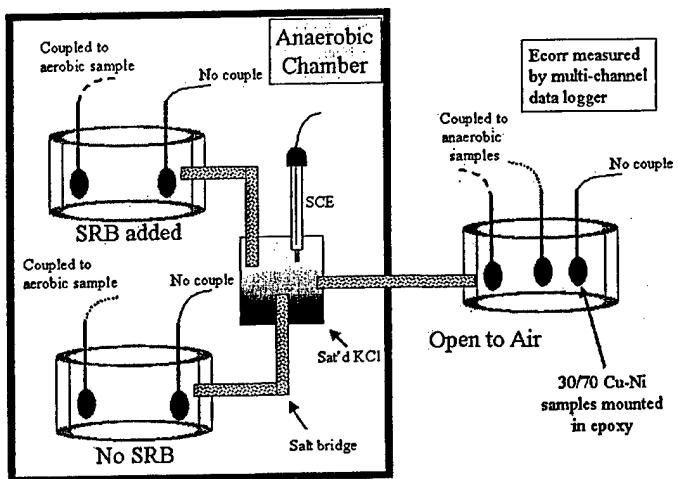


Figure 1. Schematic of the experimental set-up.

Dissolved sulfide in the SRB and uninoculated containers was maintained at the same concentration by adding Na_2S . One sample from each container in the anaerobic hood was electrically coupled (by wire) to a sample in the 'aerobic' container. After 40 hrs, bulk dissolved sulfide concentrations of the bulk solution were measured: 4.17 ppm for SRB, 0.57 ppm for uninoculated. 4 ppm Na_2S was added to uninoculated container for a final bulk concentration of 3.67 ppm sulfide. Sulfide concentrations were measured at $t = 68, 140$, and 184 hrs. After 70 hrs the coupled anaerobic samples were disconnected from the aerobic samples and left to freely corrode. At $t = 184$ hrs, all samples were removed, rinsed through a series of ASW and distilled water dilutions to remove salts. Sample surfaces were examined using an environmental scanning electron microscope (ESEM), and corrosion products were characterized by energy dispersive spectroscopy (EDS).

RESULTS

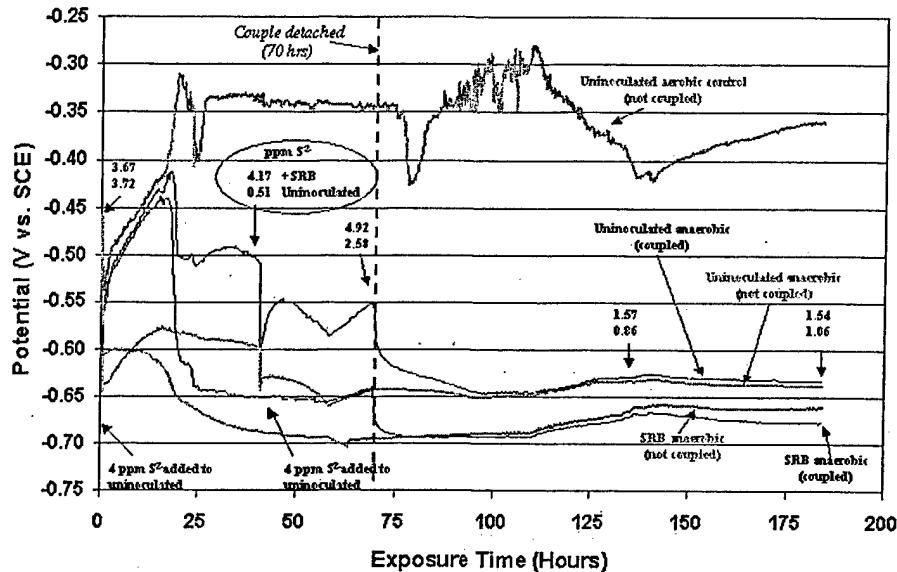


Figure 2. Potential dependence and sulfide concentrations over the course of 184 hrs.

Figure 2 indicates potential vs. time over the 184 hr experimental duration. The non-coupled sample maintained in aerobic conditions (black curve) started at ~ -550 mV and a rose to ~ -300 mV over the first 18 hours. For the duration of the experiment, potential values (black curve) ranged from -275 to -425 mV. The freely corroding (not coupled) SRB sample (blue curve) started at -600 mV, dropped to -650 mV in the first day and remained between -650 and -700 mV for the duration of the experiment. The freely corroding uninoculated sample (orange curve) started at -640 mV, rose to -570 mV over the first day, dropped to -650 mV when sulfide was added at $t = 40$ hrs, and remained stable until the end of the experiment. Both coupled samples, SRB (green curve) and uninoculated (pink curve) initially followed the potential rise of the aerobic sample (black curve) to -400 mV over the first 18 hours. At $t = 18$ hours the curves began to diverge, with the aerobic sample rising to -300 mV, the SRB sample curve dropping to -650 mV and the uninoculated sample dropping to -500 mV. At 40 hrs, the bulk dissolved sulfide concentration in the uninoculated case was increased to 3.67 ppm and potential dropped to -600 mV. Over the next 30 hrs, potential rose to -550 mV. At $t=68$ hrs, bulk sulfide concentrations of the SRB and uninoculated cases were 4.17 and 2.58 ppm, respectively. At approximately 70 hrs, the couples were disconnected and both anaerobic sample potentials immediately decreased by 50 mV. Over the next 24 hrs, the uninoculated sample continued to drop to -650 mV at which time it followed the freely corroding uninoculated sample (orange curve) to the end of the experiment, ending with potential of -640 mV. The SRB sample also followed this trend freely corroding with a final potential = ~ -660 mV. Final dissolved sulfide concentrations were 1.54 ppm (SRB) and 1.06 (uninoculated).

At the conclusion of the experiment SRB samples had very dark and uniform adherent surface deposits, with the coupled sample being the darkest. The uninoculated samples had patchy dark, non-tenuous surface deposits. In the SRB coupled case, the micrographs indicate that the surface was uniformly covered with sulfide corrosion products (Figure 3). In the uninoculated case, small patchy deposits were located on the surface. Microorganisms on the SRB sample were coated with sulfides while microorganisms on the uninoculated samples were not.

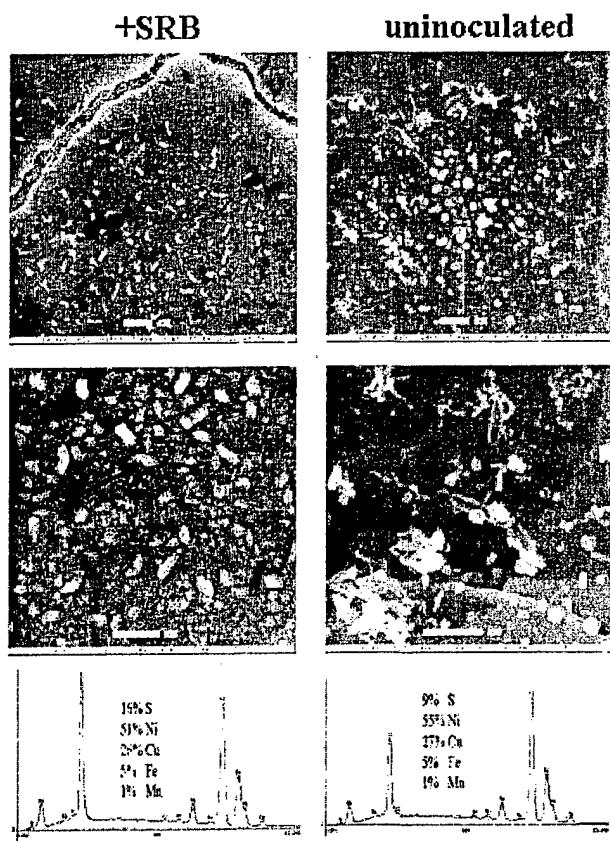


Figure 3. ESEM micrographs and corresponding EDS of surface deposits after 184 hrs.

EDS determined the SRB surface deposit to be composed of 16% sulfur, while the uninoculated deposits consisted of 9% sulfur. Similar differences were measured for uncoupled samples, 8% and 2%, respectively.

DISCUSSION

It is well established that alloy 400 is susceptible to SRB influenced corrosion.^{9, 15, 16} The process is as follows: as a result of anaerobic microbial respiration, SRB within a biofilm reduce the sulfate in seawater (2 gm L^{-1}) to sulfide. The sulfides react with the copper/nickel oxides to produce a sulfur-rich layer. Sulfide layers on alloy 400 form rapidly, causing acceleration in the corrosion rate during its formation. Maxwell¹⁷ and later Hamilton and Maxwell¹⁸ demonstrated the presence of SRB in anaerobic niches of biofilms developed in aerobic seawater. They surmised that upon exposure to oxygenated flowing seawater the sulfide layer would peel away in patches leaving bare metal exposed, thus creating an oxygen concentration cell which would provide new metal for corrosion attack. In this model, the aerated seawater supplies oxygen as a cathodic reactant to push the corrosion rate higher. However, this is often difficult to reproduce in the laboratory because of the unpredictability of sloughing, and the subsequent destruction of biofilm integrity. It was with this idea that the current authors designed this experiment in which cathodic current would be supplied remotely to a sample of alloy 400 exposed to dissolved sulfides produced by SRB. This experiment was designed to simulate the effect of oxygen on the corrosion behavior without removing the biofilm. Removal of the couple after 40 hrs (thus removing the remote cathodic current) was meant to

simulate the decrease in oxygen as a closed environment transforms from aerobic to anaerobic conditions.

Gouda *et al.*¹⁶ studied the electrochemical behaviour of copper-containing alloys in seawater exposed to sulfides and SRB. Using polarization resistance (R_p) and anodic polarization scans, they demonstrated that passivation of the metal surface occurred upon initial exposure of alloy 400 to an SRB environment. As seen in Figure 2, ennoblement of the coupled samples followed the rising potential of the aerobic sample over the first 18 hrs. While these data may indicate passivation of the alloy 400 surface, they more likely indicate the coupled samples were cathodically controlled over this time (by the aerobic electrode), corresponding with the build-up of dark surface deposits seen on both SRB and uninoculated surfaces in the first 18 hours. This process seems to be independent of SRB. However, at 18 hrs, differences are observed. The SRB potential drops from -440 mV down to -650 mV while the uninoculated potential drops from -410 mV only down to -500 mV. The difference in magnitude for the potential drops between SRB and uninoculated cases is possibly due to the formation of a patchy SRB biofilm which allowed the sulfide concentration at the metal/biofilm interface to increase, thereby, decreasing the potential. In contrast, the uninoculated case which did not contain intentionally introduced SRB, would not decrease in potential as much due to the lower sulfide concentration. Bulk sulfide concentration increasing over the first 40 hrs in the SRB case indicated the presence of growing SRB and the presence of a dark surface film indicates the incorporation of sulfide into the surface oxide. However, dissolved sulfide concentration in the uninoculated case declined steeply over the first 40 hrs indicating sulfide was not being produced. Throughout the experiment, the potential of the coupled SRB sample was always lower than the coupled uninoculated sample. This trend was also observed in the freely corroding samples.

Differences in sulfides produced by bacteria within biofilms and waterborne inorganic sulfides were identified. Sulfide layers formed in biofilms during exposure to SRB covered the entire surface of the sample. In contrast, exposure to uninoculated ASW and inorganic sulfides resulted in only localized sulfide deposits covering a fraction of the metal surface. Sulfide layers formed in biofilms were also more tenacious than those formed in the uninoculated case. The tenacity of the SRB sulfide layers may be due to the extracellular polymeric substances (EPS) produced within the SRB biofilm. EPS may act as an adhesive that thereby strengthens the sulfide layer against sloughing. Lee *et al.*¹⁹ found similar results in the corrosion of alloy 400 in the presence of SRB.

Chemical composition of sulfide layers produced by exposure to uninoculated and SRB containing seawater also differed. In the case of freely corroding samples, the SRB corrosion products had a high sulfur concentration of 8%, while uninoculated products were composed of only 2% sulfur. In the case of the coupled samples, the sulfur concentration increased to 16 and 9% for the SRB and uninoculated cases, respectively. The higher concentration of sulfur in the SRB containing media is probably due to the production of sulfides at the biofilm/metal interface. The higher concentration of sulfur due to coupling can be attributed to a combined affect of increased reactivity at the metal surface due to a driving cathodic current, increased activity of metal ions bound to SRB (see below) and the attraction of bacteria to the anodic electrode by electrostatic forces.²⁰ Sulfur concentration in the SRB layer is especially high considering the bulk solution had a sulfide concentration of ~4 ppm. These findings indicate a connection between bacterial activities and the resulting surface morphology. Active bacterial surface-mediated mineralization occurs either by the direct transformation of metals (i.e., methylation, redox reactions) or by the formation of metal-reactive by-products (i.e., sulfate reduction producing sulfide). Reduced iron and other metal ions are commonly precipitated on dissimulatory SRB cell surfaces as sulfides.²¹

Gouda *et al.*^{9,15} examined the susceptibility of alloy 400 towards microbial attack in Arabian Gulf seawater. Results indicated that SRB attack is initiated beneath black sulfur-rich deposits. The deposits were found to be mostly iron/nickel sulfides. No corrosion was detected after 3 weeks of exposure under anaerobic SRB conditions, but upon addition of aerated solution, the corrosion rate increased significantly.

CONCLUSIONS

Distribution, tenacity and chemical composition of sulfides produced by SRB within biofilms are different from those produced by waterborne inorganic sulfides. In an anaerobic environment, SRB within biofilms produce sulfides at the metal surface that lead to an increased sulfur in corrosion products. Coupling to an external cathode exposed to aerobic conditions increase the sulfur content further. Because bacteria enmeshed in biofilms produce extracellular polymeric materials the tenacity of sulfide layers produced by SRB may differ from those produced by inorganic reactions. In the experiments described, only the samples exposed to SRB had encrusted bacteria embedded within the sulfide layer.

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